BioTechniques **15**:856-862 (1993)), is included in the vector as a cis-acting inducer of gene expression. This sequence enhances production of GFO. Other leader sequences may be substituted for ATL.

(k) *gfo*. A hybrid *gfo* gene is included in the vectors. This gene is composed of a mutant GFP at the 5' end and neomycin (NEO) coding sequences at the 3' end. GFP mutants are derivatives of the *Aequorea victoria* GFP, an autofluorescent protein widely used as a reporter of gene expression (Chalfie et al., Science **263**:802-805 (1994); and Palm et al., Nature Structural Biology **4**:361-365 (1997)). Preferred mutants encode a green fluorescent protein with increased cellular fluorescence and include, without limitation, a GFP sequence which is based on the sequence of Heim et al. (Current Biology **6**:178-182 (1996)) but which includes at least one of the following mutations: P4-3 (Y66H, Y145F), W7 (Y66W, N146I, M153T, V163A, N212K), SG11 (F64L, I167T, K238N), SG25 (F64L, S65C, I167T, K238N), or SG50 (F64L, Y66H, V163A). The gfo sequence is used for fluorescence activated cell sorting (FACS) of infected ES cells, an important step in the generation of LOKs. The *neo* gene codes for bacterial neomycin phosphotransferase (Southern and Berg, J. Mol. Appl. Gen. **1**:327-341 (1982)). Expression of this sequence renders ES cells which contain the provirus resistant to G418. Neomycin resistance is used in the methods of the invention to select ES cells which are homozygotic for the proviral insertion; this is accomplished by increasing the concentration of G418 in the cell culture medium, as previously described (Mortensen et al., Mol. Cell. Biol. **12**:2391-2395 (1992)). Other detectable and selectable markers may also be utilized in the invention.

(l) SPA. A synthetic polyA addition signal is also included in the vector to facilitate processing and expression of the *gfo* mRNA (Levitt et al., Genes Dev. **3**:1019-1025 (1989)). Other synthetic or natural poly A sequences may be utilized.

(m) t. Transcriptional termination sequences are an important feature of the retroviral vectors. These sequences terminate transcription from both the PGK and the

cellular promoters. Appropriate transcription termination results in a considerably increased mutagenic potential of the retroviral insertion and a decrease in the abnormal expression of genes adjacent to the provirus; this eliminates potential complications in the phenotypic characterizations of KO mice, as has been observed in some instances (Olson et al., Cell **85**:1-4 (1996)). As shown in Figure 1, a preferred termination sequence is derived from the human complement gene (Ashfield et al., EMBO J. **10**:4197-4207 (1991)), but any other appropriate transcription termination sequence may be utilized.

(II) Unique Properties of and Uses for the Retroviral Vectors of the Invention

The vectors of the invention possess a number of unique properties, making them useful for various types of gene disruption methods and types of analyses. Examples of these unique properties and uses now follow.

The retroviral vectors are highly mutagenic. One significant advantage provided by the present retroviral vectors is the fact that these vectors are highly mutagenic. This property arises, at least in part, because the vectors contain a combination of a consensus splice acceptor and transcriptional termination sequences. The splice acceptor has been previously described (Gossler et al., Science **244**:463-465 (1989); Friedrich and Soriano, Genes Dev. **5**:1513-1523 (1991); Skarnes et al., Genes Dev. **6**:903-918 (1992); Takeuchi et al., Genes Dev. **9**:1211-1222 (1995); Wurst et al., Genetics **139**:889-899 (1995); Forrester et al., Proc. Natl. Acad. Sci. USA **93**:1677-1682 (1996); and Brenner et al., Proc. Natl. Acad. Sci. USA **86**:5517-5521 (1989)), but the combination with termination sequences is novel, and this combination is important for the elimination of read-through transcription which is frequently observed in cellular sequences flanking proviruses (Swain and Coffin, Science **255**:841-845 (1992)). The termination sequence also enhances mutagenicity by blocking potential bypassing of the insertion by alternative splicing mechanisms which make use of fortuitous chromosomal splice sites; these sites

are inaccessible due to transcription termination at t.

Insertion of the retroviruses into a gene of interest, for example, gene X in Figures 2-4, leads to gene inactivation which is independent of the site of integration. Normal transcription and subsequent translation of gene X (Fig. 2) are disrupted, whether or not the retroviral insertion has occurred in an exon sequence (Fig. 3) or an intron sequence (Fig. 4). This advantage is quite important. Although gene disruption is generally expected following integration of standard retroviruses into exons, the outcome of retroviral integration into introns is less predictable, and only a small fraction of retroviral insertions have been found to be associated with recessive phenotypes in the mouse (Jaenisch, Science **240**:1468-1474 (1988)). Accordingly, the combination of a splice acceptor sequence and a transcriptional terminator is an important feature of the present invention, rendering the presently described vectors highly mutagenic even when integrated at intron locations.

The MAGEKO method allows rapid identification of infected cells. In a second advantage, the invention allows rapid identification of infected cells. As described above, the vectors of the invention include a marker which facilitates the identification of vector-containing cells. In one embodiment, the vectors carry a GFP mutant with increased cellular fluorescence linked to the PGK promoter. This marker allows for the identification of infected cells hours after infection, thus enabling the rapid sorting of transduced cells, for example, by FACS analysis. This is an important element for the generation of LOKs.

The MAGEKO approach provides for specific detection of cells expressing the mutant gene. As described above, the fusion gene, rtTA, is produced only in cells expressing the gene mutated by a retroviral insertion. The conditional nature of rtTA synthesis allows the specific tagging of insertion-containing cells through a binary mammalian system, such as a binary mouse system. According to this technique, mice carrying the retroviral vector of the present invention may be mated to mice containing a marker gene under the